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PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Mark Brader, *et al.*	
Serial No.: 08/484,542	
Filed: June 7, 1995	Group Art Unit: 1631
For: **Stabilized, Acylated Insulin Formulations**	Examiner: M. Allen
Docket No.: X-10097	

DECLARATION OF DR. MARK L. BRADER UNDER 37 C.F.R. § 1.608(b)

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Mark L. Brader declares as follows.

1. I received my Ph.D. in Chemistry from Massey University. I completed two postdoctoral fellowships, one at the University of California in 1988-1991, and one at Rutgers University in 1992-1993 year. I have been employed by Eli Lilly & Company as a product development scientist since May 24, 1993. I currently hold the title of Research Scientist at Eli Lilly & Company.

2. I am a co-inventor of the subject matter encompassed by the claims of the above-referenced patent application.

3. I make this declaration to establish the dates of conception and reduction to practice of a composition comprising an aqueous solution of a fatty acid-acylated insulin and zinc.

4. I conceived of and made a composition comprising an aqueous solution of LY309132 and zinc on or before September 9, 1993. LY309132 is a human insulin derivative that is acylated with a 16 carbon fatty acid (palmitic acid) at the epsilon amino group of lysine at position B29. In this declaration, I refer to this compound as C16-insulin.

5. Exhibit 1 is a photocopy of pages 39 through 41 of my laboratory notebook number 2685 and describes work I performed to show the suitability of a C16-insulin zinc formulation for use in a clinical setting. I remember doing the work described on these pages. Further, the handwriting on these pages is my handwriting and the signature at the bottom of each page is my signature. The work described on these pages was performed on September 8, 1993 through September 10, 1993 as indicated by the date written at the top of page 39 and the bottom of pages 40 and 41 of my notebook.

6. The work documented in Exhibit 1 verified that zinc does not adversely affect the solubility properties of C16-insulin, and that a lyophilized powder containing C16-insulin and zinc can be readily reconstituted with an appropriate diluent in a clinical setting.

7. The composition set forth and described in Exhibit 1 was prepared as follows:

A 112.4 mg quantity of C16-insulin from lot number DBF40 was dissolved in 18 mls of 0.01 N HCl and divided into two 9.050 ml samples. One sample was used to prepare a composition

without zinc, and one sample was used to prepare a composition with zinc.

The zinc composition is referred to as DBF40Zn in Exhibit 1. This composition was prepared by adding 60.9 µl of a 3.91 mg/ml ZnO stock solution to one of the 9.050 ml samples containing C16-insulin described above. The pH of the zinc containing aqueous solution was adjusted to 7.42 by adding 1040 µl of 0.2 M NaOH. Water (100 µl) was added to bring the final volume of the solution to 10.25 mls.

Thus, the resulting composition was an aqueous solution with a pH of 7.42 containing approximately 4.93 mg/ml of C16-insulin (assuming 90% potency of the C16-insulin solid) and zinc at a molar ratio of 0.35 (zinc to C16-insulin).

8. I wrote in page 40 of my notebook that the solution remained clear after the addition of zinc. Thus, the presence of zinc did not appear to adversely affect the solubility of C-16 insulin in the composition.

9. On September 9, 1993, I provided the composition described in Paragraph 7 hereof to Michael Roy for freeze drying so that I could confirm that a lyophilized C16-insulin zinc formulation would dissolve adequately and that the C16-insulin would remain soluble upon reconstitution.

10. I received the lyophilized powder from Michael Roy on September 10, 1993. I reconstituted the powder by adding 1 ml of Humulin R diluent. Humulin R is a commercially available human insulin sold as a solution formulation. Humulin R diluent is an aqueous solution containing: 16 mg/ml glycerin and 2.5 mg/ml m-cresol. Several aliquots were reconstituted. In each sample, the lyophilized C16-insulin

dissolved completely in less than 30 seconds yielding a clear solution.

11. The clarity of the reconstituted aqueous zinc containing solution and the short time needed for complete reconstitution confirmed the suitability of a lyophilized zinc powder for use in a clinical setting. I expected the C16-insulin to be readily absorbed when injected into an animal or human subject because of the clarity of the aqueous solution both before lyophilization and after lyophilization upon reconstitution in diluent. Further, since the C16-insulin would be expected to be absorbed, I also expected it to exert a hypoglycemic effect *in vivo*.

12. On September 28, 1993, I prepared a scaled-up version of the composition described in Paragraph 5 hereof. As expected, the scaled-up C16-insulin lyophilized powder containing zinc rapidly dissolved upon reconstitution with Humulin R diluent further confirming its utility as a formulation appropriate for use in the clinic.

13. Exhibit 2 is a photocopy of pages 47 and 48 of my laboratory notebook number 2685. I remember doing the work described on these pages. Further, the handwriting on these pages is my handwriting and the signature at the bottom of each page is my signature. The work described on these pages was performed on September 28, 1993 as indicated by the date written at the top of page 47 and the bottom of pages 47 and 48 of my notebook.

14. As shown in my notebook, the scaled-up solution was prepared as follows:

A 1.20 g quantity of C16-insulin from C16-insulin lot number 487EM3 was dissolved for 30 minutes in 150 mls of 0.01 N HCl and divided into two 75 ml aliquots.

The zinc-containing composition was called lot 2685-47A, and was prepared by adding 1 ml of a 2.4 mg/ml ZnO stock solution to one 75 ml sample containing c16-insulin described above. The pH of the zinc containing aqueous solution was adjusted to 7.6 with NaOH and the final volume was adjusted to 100 mls with H_2O. This aqueous solution was provided to Michael Roy at 4:00 PM on September 28, 1993 for freeze drying.

15. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information of belief are believed to be true; and I am warned that all statements made herein were made with the knowledge that willful false statements are punishment by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful and false statements may jeopardize the validity of any patent issued from this application.

Mark L. Brader

Mark L. Brader

4/23/03

Date